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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

BARCHFELD et al.

Serial No.: 09/044,696 Art Unit: 1641

Filing Date: March 18, 1998 Examiner: S. Devi

Title: DETOXIFIED MUTANTS OF BACTERIAL ADP-RIBOSYLATING TOXINS AS PARENTERAL ADJUVANTS

DECLARATION UNDER 37 C.F.R. § 1.132

I, Giuseppe del Giudice, declare as follows:

1. I received my M.D. at the University of Milan in 1980 and a specialization in Infectious Diseases in 1984 from the University of Milan.

2. I have been employed by the Research Center of Chiron Vaccines, in Siena, Italy since 1996 and hold the position of Research Director. I am currently responsible for vaccine projects involving detoxified cholera toxin (CT) and *E. coli* heat-labile toxin (LT) ADP ribosylating toxins as well as other vaccine projects. I am extremely familiar with studies of detoxified mutants and with adjuvants having actively studied and worked in this discipline for over 15 years. I have co-authored numerous publications and patents in the field of immunology, including publications relating to detoxified CT and LT mutants. A copy of my Curriculum Vitae (Exhibit A) is attached hereto.

3. I have reviewed relevant documents from the prosecution of the above-referenced application (hereinafter "the application"), including the Office Action dated April 25, 2000 and the art cited therein, in particular, WO 97/02348 (hereinafter "WO

'348"); WO/18928 (hereinafter "WO '928"); and Pizza et al. (1994) *Mol. Microbio.* 14:51-60 (hereinafter "Pizza et al.").

4. I understand that the claims pending in this application are directed to methods of immunizing vertebrate subject by parenteral administration of an adjuvant and selected antigen. The parenterally administered adjuvant includes a detoxified mutant of *E. coli* LT ADP-ribosylating mutant (*e.g,* LT-R72 or LT-K63) toxin and a pharmaceutically acceptable vehicle. I further understand that, as used in the application, the term "detoxified" refers both completely non-toxic and low residual toxic mutants (*e.g.,* less than 0.01% of the naturally occurring toxin counterpart). Toxicity is determined, for example, using the well known YI assay. In addition, I understand that, as used in the application, the term "parenteral" refers to introduction into the body outside of the digestive tract and excludes introduction to mucosal surfaces. Thus, I am familiar with the invention and the issues raised by the Office.

5. I believe that one working in this field would clearly consider both LT-R72 and LT-K63 to be "detoxified" mutants. As noted above in paragraph 4, the application defines "detoxified" as mutants having less than 0.01% toxicity of the naturally occurring toxin, for example as measured by the art-recognized YI assay. Example 1 indicates that LT-K63 is inactive "*in vitro* on YI cells." (see, page 28, lines 14-15 of the application). Similarly, LT-R72 is also detoxified, as evidenced by Figure 4 in WO 98/18928 which shows that LT-R72 has much less than 0.01% the toxicity of the naturally occurring toxin. Therefore, both LT-R72 and LT-K63 are detoxified mutants.

6. It is further my opinion that one skilled in the art would understand from the application that the claimed detoxified mutants adjuvants could be administered using any known mode of parenteral administration. Again, as noted above in paragraph 4, the application defines "parenteral administration" as non-mucosal administration outside the digestive tract of the subject. The application discusses how to prepare injectable compositions, topically applied compositions, gel formulations, and cites references

providing additional parenteral administration techniques. (see, *e.g.*, pages 22-24 of the application and references cited therein). Furthermore, subcutaneous, transcutaneous and intramuscular routes are specifically demonstrated in the Examples of the application. Thus, I believe that, based on the application and level of skill in the art, one working in this field would be able to immunize a subject with the claimed detoxified adjuvants using any mode of parenteral administration.

7. It is also my opinion that WO '348 does not describe or demonstrate the methods claimed in the application. First, WO '348 does not disclose methods of immunization using the claimed detoxified mutants as adjuvants. Rather, WO '348 is primarily focused on use of detoxified mutants as antigens (see, for example, claim 3 directed to vaccines comprising the detoxified mutant and an adjuvant and claim 4 directed to vaccines comprising the detoxified mutant and a second immunogenic agent). Adjuvanticity is not tested. Second, parenteral administration of the claimed detoxified mutants is not described or demonstrated by WO '348. The immunogenicity of these mutants is described only in mucosal immunization regimes. Certainly, there is no description or suggestion that the claimed mutants could be used as parenteral adjuvants. Accordingly, I do not believe that WO '348 in any way teaches the use of LT-R72 or LT-K63 as parenteral adjuvants.

8. It is further my opinion that Pizza et al. is not relevant to the subject matter claimed in the application. Pizza et al. is concerned with analyzing the structure-activity relationship of LT-A mutants. One working in this field would have no reason to apply this information to use of LT mutants as parenteral adjuvants. In particular, Pizza et al. gives LT-R72 a "++" rating in terms of toxicity on YI cells. This is compared to a "+++" rating for wild type. Although, Pizza does not provide absolute values, it is my opinion that one working in this field would not consider a rating of "++" to be "detoxified" as defined in the application. Accordingly, I do not believe that Pizza is relevant to the claimed invention.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: × 17 October 2000 Signature: ×

Giuseppe del Giudice, M.D.

CURRICULUM VITAE

Giuseppe Del Giudice, M.D.

PERSONAL DATA

Date of birth:	16 July 1956
Place of birth:	Apricena, Italy
Citizenship:	Italian
Marital status:	Married with Teresa Pedrotti, Italian (14 December 1985); two children, Eloisa (12 November 1987) and Federico (21 March 1989)
Mother tongue:	Italian
Other languages:	Fluent in English and French (spoken and written)
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EDUCATION

Undergraduate:	Liceo-Ginnasio Statale "G. Carducci", Milan, Italy. Diplome, 1974.
Graduate:	Doctor in Medicine and Surgery, Faculty of Medicine and Surgery, University of Milan, Milan, Italy, September 1980. Medical Board, November 1980
Postgraduate:	• Specialization in Infectious Diseases, Faculty of Medicine and Surgery, University of Milan, Milan, Italy, October 1984. • Doctor in Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland, October 1989

PROFESSIONAL RECORD

1980-1984:	Post-doctoral Fellow, Clinica Medica II, University of Milan, Milan, Italy
1985-1986:	Research Assistant, WHO-Immunology Research and Training Centre, Department of Pathology, University of Geneva, Geneva, Switzerland
1987-1993:	Research Associate, head of laboratory, WHO-Immunology Research and Training Centre, Department of Pathology, University of Geneva, Geneva, Switzerland
1989-1993:	WHO Medical Officer, Microbiology and Immunology Support Services, Division of Communicable Diseases, WHO Headquarters, Geneva, Switzerland
Oct. 1992:	Privat-Docent (Agrégé), Faculty of Medicine, University of Geneva, Geneva, Switzerland

Jan 1994:-Mar 1996	• Maître-Assistant, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland • Ajoint scientifique (Research Officer), Office of the Dean, Faculty of Medicine, University of Lausanne, Lausanne, Switzerland
Mai 1995	Privat-Docent (Agrégé) , Faculty of Medicine, University of Lausanne, Lausanne, Switzerland
From Apr. 1996	Research Director, Department of Immunology, Research Center of Chiron Vaccines, Siena, Italy.

MEMBERSHIPS

- Italian Society for Immunology and Immunopathology (1980-1982)
- Italian Society for Parasitology (since 1986)
- Swiss Society for Immunology (since 1986)
- American Association of Immunologists (since 1989)
- Itlian Society of Immunology (since 1989)

PUBLICATIONS

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